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INDIVIDUAL-CELL BASED MODEL FOR CHARACTERIZING THE MECHANICAL MICROENVIRONMENT IN MICROCARRIER CELL EXPANSION

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INTRODUCTION

Tissue Engineering applications require the availability of three-dimensional cell culture systems which offer a well-controlled cell environment and can accommodate a large number of cells [1]. In microcarrier systems, high densities of cells can be seeded on spherical microbeads, which can be clustered in order to create large three-dimensional cell aggregates [2]. The biophysical properties of the microcarrier determine the physical microenvironment of the cells. The microenvironment should have well-adjusted mass transport properties, as well as offer a proper mechanical environment for the cells [3].

In this study we demonstrate a framework for individual-cell based models (IBMs) for quantifying the relationship between design characteristics of microbeads and the mechanical microenvironment to which individual cells are exposed. The robustness of the model is characterized in a sensitivity analysis and the influence of process design and cell culture dependent parameters on the magnitude and heterogeneity of mechanical stresses is characterized.

METHODS

A lattice-free IBM is used which considers cells as deformable spherical particles. At each time point, the displacement of the cells is calculated from the equation of motion, which is derived for cells that move in a low-Reynolds number environment [4]. Summing up body forces and contact forces between next neighboring particles i and j yields:

$$\sum_{jnni} \boldsymbol{F}_{ij} + \boldsymbol{F}_{i,s} + \boldsymbol{F}_{i,\text{division}} + \boldsymbol{F}_{i,\text{Brownian}} = \Gamma_{iw}\boldsymbol{v}_i + \Gamma_{is}\boldsymbol{v}_i + \sum_{jnni} \Gamma_{ij}(\boldsymbol{v}_i - \boldsymbol{v}_j) \quad (1)$$

in which all velocity dependent forces are summed up at the right hand side: a viscous drag force, cell-carrier and cell-cell friction forces.

The mechanical behavior of a cell is largely determined by the properties of the cytoskeleton, which will actively adapt to external force and exert pulling forces on anchoring points of the environment. In the IBM, cells are considered deformable elastic spheres with a contact area dependent force described by the Johnson-Kendall-Roberts (JKR) potential. The combination of elastically stored energy in the JKR potential and energy dissipation through drag forces determines the overall cell mechanical stress which changes due to micro-environmental conditions.

In the IBM, it is sufficient to describe the cell cycle of growing cells from a purely morphological perspective. Therefore, the cell cycle is split into two distinct phases: cell volume growth (interphase) and cytokinesis (during mitosis). During the interphase, the rate of volume increase during time is considered constant. Cytokinesis is approximated using a dumb-bell description of two overlapping spheres [4]. During cytokinesis, it is assumed that the cytoplasmic volume remains constant.

Simulations were performed for standard conditions for cell expansion on spherical non-porous microbeads. The mechanical micro-environment is quantified by means of the hydrostatic pressure P. Positive values of P indicate compressive stress on the cells, while negative values indicate tensile stress.

For the sensitivity analysis, a latin hypercube experimental design is used that resulted in 100 samples, i.e. 100 simulations. Cells are randomly seeded on the microcarrier at a fixed seeding density, and exponential cell growth is simulated until confluency is reached. Before and after confluency, two quantities are calculated as output variables: the mean compressive mechanical stress and the standard deviation on the compressive stress (a measure for stress heterogeneity).

RESULTS

The sensitivity analysis quantifies how small changes in input parameters affect the predicted mechanical stress. In order to offer predictive value, the theoretical system should not result in a strong variation of mechanical stresses for small variations of input parameters. The uncertainty analysis indicates the global sensitivity of the model:

Table 1 Uncertainty analysis for 100 simulations: expected value and variance on mean mechanical stress and variance at confluency

	Expected value (E)	Variance (V)
$\bar{P}$	0.2153	0.0088
H_p	0.6393	0.0267

Both for P and H_p, the standard deviation (or $V^{1/2}$) is significantly lower than the expected value, indicating robust model behavior. To further quantify model sensitivity, a linear model is constructed that describes the predictions of mechanical stress as a linear combination of model input parameters (Table 2).

Table 2 Standardized coefficients of linear model. Data is time-averaged after confluency

i	Coefficient	Estimate (a_i)	Std. Error	T value	Pr ($>\|t\|$)	$p < 0.001$
0	Intercept	1.392e-16	6.753e-02	0.000	1.000	
1	E_c	-3.191e-01	7.338e-02	-4.348	8.56e-05	*
2	$\gamma_\perp$	-7.386e-02	7.251e-02	-1.019	0.314	
3	$\gamma_\parallel$	2.841e-02	6.829e-02	0.416	0.679	
4	$K_{a,cc}$	7.813e-01	7.266e-02	10.753	1.23e-13	*
5	$K_{a,cb}$	1.111e-01	6.956e-02	1.597	0.118	

For the data after confluency, significance was found for cell stiffness and cell-cell adhesion energy. In other words, predicted stress values are most sensitive to cell-line dependent mechanical parameters.

Simulations were performed for different levels of cell-bead adhesion energy (Figure 1 and 2). Higher values of cell-bead adhesion energy lead to higher mean compressive stress values and a higher rate of stress increase around the point of confluency.

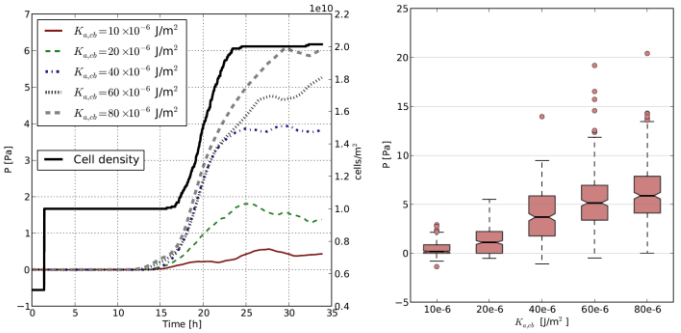

Figure 1: left: Temporal evolution of mean stress level for different cell-bead adhesion values. **Right:** Boxplot indicating stress levels calculated after confluency. Statistically, the pairwise difference between the group means of any combination is significant ($p < 0.001$).

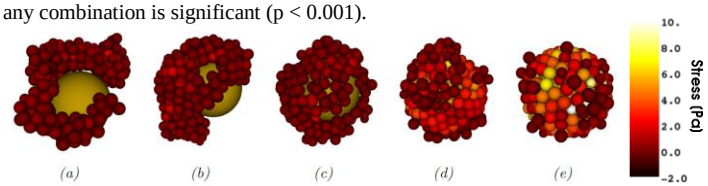

Figure 2 Mechanical stress on cells on microcarrier at confluency for different cell-substrate adhesion values (K_a). **a**: K_a = 2.5e06 J/m2, **b**: K_a = 5e-6 J/m2, **c**: K_a = 10e-6 J/m2, **d**: K_a = 20e-6 J/m2, **e**: K_a = 40e-6 J/m2

Seeding cells with synchronized cell cycles will result in a lot of cytokinesis events initiating at the same time. The influence of cell cycle synchronization on the stress values was examined by varying the standard deviation σ on the initial radius of the cells (Figure 3). No significant difference was found for the mean mechanical stress, but stress heterogeneity is significantly higher for completely asynchronous cell cycles.

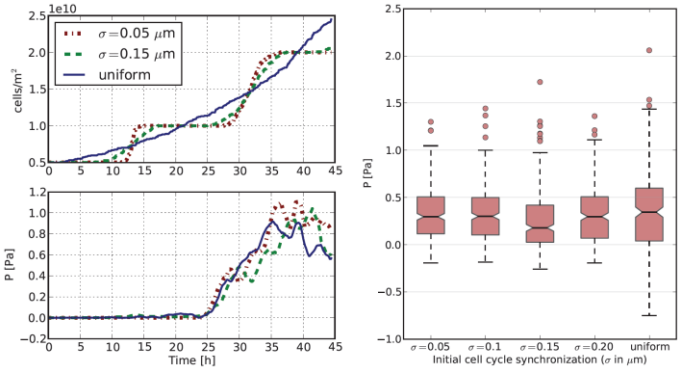

Figure 3 Influence of cell cycle synchronization. **top**: temporal evolution of cell number with σ standard deviation on initial cell radius**, bottom**: temporal evolution of mean stress level **right**: boxplot of stress values after confluency.

DISCUSSION

In this study an IBM was developed that describes cell expansion on non-porous spherical microcarriers. The model describes the temporal behavior of mechanical stresses for growing cells and provides a theoretical system that can be used to characterize the influence of process design parameters on the mechanical micro-environment. Simulations revealed that the cell-bead adhesion energy, which is influenced by the adhesive coating applied to the carrier, is a strong determinant for the magnitude of mechanical stresses on the cells. Furthermore, larger bead sizes result in higher values of mechanical stress on the cells. The level of cell cycle synchronization does not strongly influence the magnitude and heterogeneity of the mechanical microenvironment.

In general, the simulations give a proof of principle that a large biological heterogeneity is not required to result in a strongly heterogeneous mechanical microenvironment. Purely spatial and mechanical properties of the carrier system can result in localized differences in mechanical stress on the cells. Because cell behavior and cell fate have been shown to be strongly influenced by structural and mechanical phenomena, it can be hypothesized that a large phenotypic heterogeneity might arise regardless of the biological heterogeneity of the seeded cell pool.

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